

The mutation rate of codon 43 was 57.5% (61/106). The results of HRM of the 50 strains were identical to those of sequencing.

Conclusion: Mutation in *rpsL* gene is the important molecular mechanism of streptomycin resistance in *M.tuberculosis*. High-resolution melting method is a sensitive in-tube methodology to screen for mutations of streptomycin resistance in *M.tuberculosis*. HRM analysis using a dsDNA dye provides a useful tool and is applicable for rapid and high-throughput screening of *rpsL* gene mutations in *M.tuberculosis*.

Free Paper Presentation 2 – Hepatitis B

OL-010 Biochemical, immunological and virological profiles of, and differential diagnosis between patients with acute hepatitis B and chronic hepatitis B with acute flare

Y.N. Han, Q. Tang, W. Zhu, X.Q. Zhang, L.Y. You. *Research Unit of Liver Disease, Shanghai No. 8 People's Hospital, Shanghai 200235, China*

Background and Aim: In areas of intermediate or high prevalence of chronic hepatitis B virus (HBV) infection, it is difficult to distinguish acute hepatitis B (AHB) from chronic hepatitis B with an acute flare (CHB-AF) in patients whose prior history of HBV infection was unknown. The Immunoglobulin M antibody against hepatitis B core antigen has been used as a differentiation indicator. The present study aimed to screen other laboratory parameters to construct a more efficient joint diagnosis method.

Methods: A retrospective and prospective study was carried out in patients first clinically presenting as HBV-related acute hepatitis to sort out the individuals with acute self-limited hepatitis B (ASL-HB). Laboratory testing profiles were compared between patients with ASL-HB and CHB-AF. The sensitivity, specificity, accuracy, positive predictive values, and negative predictive values for diagnosing AHB of the parameters closely associated with ASL-HB were evaluated.

Results: Significant differences were observed between patients with ASL-HB and CHB-AF in laboratory profiles, and the differences showed in the increased levels of serum HBV DNA, HBV e antigen (HBeAg), alpha-fetoprotein (AFP) and anti-HBc IgM. Furthermore, It was found that the population number of ASL-HB patients was significantly higher than that of CHB-AF in those with low levels positive HBeAg ($S/CO < 20$) (42.7% vs 13.5%), but this was not found in HBeAg negative group (49.3% vs 45.9%). For predicting ASL-HB, the sensitivity and specificity of single 1:10 000 anti-HBc IgM were respectively 96.2% and 93.1%. Combining anti-HBc IgM with HBV DNA, or and HBeAg could improve the diagnostic power. The combination of anti-HBc IgM, HBV DNA, and HBeAg had a positive predictive value of 98.9% and a negative predictive value of 100.0%, similar to those of the combination of IgM anti-HBc and HBV DNA. AFP added to either combination groups further increased the positive predictive value. Both the positive predictive value and negative predictive value of the combination of IgM anti-HBc, HBV DNA and AFP were 100.0%.

Conclusions: (1) There are significant differences in biochemical, immunological and virological aspects between ASL-HB and CHB-AF. (2) Among several joint diagnostic methods, IgM anti-HBc combining HBV DNA and AFP is the most effective and practical in distinguishing ASL-HB from CHB-AF. (3) A low HBeAg level is more helpful than negative HBeAg in differential diagnosis between ASL-HB and CHB-AF.

(4) Patients with a high level of IgM anti-HBc, serum AFP level $>5 \times \text{URL}$ could rule out a probability of ASL-HB.

OL-011 Positive serum hepatitis B e antigen associated with higher risk of early recurrence and poorer survival in patients after curative resection of hepatitis B-related hepatocellular carcinoma

H.C. Sun¹*, W. Zhang¹, L.X. Qin¹, B.H. Zhang¹, Q.H. Ye¹, L. Wang¹, N. Ren¹, P.Y. Zhuang¹, X.D. Zhu¹, J. Fan¹, Z.Y. Tang¹. ¹*Liver Cancer Institute and Zhongshan Hospital, Fudan University, Shanghai, China*

Background and Aim: To study the impact of hepatitis B e antigen on tumor recurrence and patients' survival after curative resection of hepatocellular carcinoma.

Methods: 203 patients with small hepatocellular carcinomas (≤ 3 cm) undergone curative resection were divided into HBeAg-positive group and HBeAg-negative group. Clinicopathological factors and postoperative outcomes were compared between groups, and risk factors for survival and recurrence were studied.

Results: The median follow-up time is 32.9 months. Those with negative HBeAg were better in both 5-year overall survival (76% vs 53.9%, hazards ratio (HR): 2.363, 95% CI: 1.335–4.181, $P=0.002$) and 5-year disease-free survival (52.9% vs 37.4%, HR: 1.603, 95% CI: 1.00–2.561, $P=0.046$). There was no significant difference in operative morbidity and tumor factors between the two groups, but younger age, higher serum alanine aminotransferase level, and more macronodular cirrhosis were found in HBeAg-positive group. Multivariate analysis revealed that age >50 years, HBeAg positivity and macronodular cirrhosis were independent factors for overall survival, and HBeAg positivity and multiple tumor nodules were independent factors for disease-free survival. Positive serum HBeAg associated with a higher risk of early recurrence (within 1 year).

Conclusions: Positive HBeAg associated with a higher risk of early recurrence and poorer survival of patients after curative resection of small HCC.

OL-012 HepG2.2.15 can induce the expression of PD-1 on T-cell: may be a mechanism of immune tolerance in HBV infection

J. Chen*, X.J. Wu, Y.H. Wang, Y. Wang, J. Li, M. Yu, N.L. Zhang, G.Q. Wang. *Department of Infection Disease, Peking University First Hospital, Beijing 10034, China*

Background and Aims: To investigate the effect of HepG2.2.15 – a cell line transfected HBV genome in inducing PD-1 (Programmed cell death-1) expression on T-cell, and the function of PD-1 on T-cell.

Methods: HepG2.2.15 were co-cultured with Jurkat cells, which a kind of T lymphocyte. The PD-1 expression was detected by flow cytometry (FCM); Blocking test by adding anti-PD-1 antibody in the experiment; Cytokines in culture supernatant of blocking groups and control groups were measured by enzyme-labeled immunosorbent assay (ELISA); Cytotoxic test of T-cells in blocking groups and controls were measured by methyl thiazolyl tetrazolium (MTT).

Results: The PD-1 expression on T-cells was induced by HepG2.2.15, the expression rate is $17.43 \pm 5.6\%$ (control is $1.22 \pm 0.2\%$); The cytokines IL-2 level (202.9 ± 53.0 pg/ml), INF- γ level (88.6 ± 4.6 pg/ml) and IL-10 level (63.7 ± 13.4 pg/ml) in culture supernatant of blocking groups were significant higher than that of controls (IL-2, 102.9 ± 53 pg/ml, INF- γ , 39.3 ± 4.2 pg/ml and IL-10, 34.6 ± 13.7 pg/ml, respectively. $p < 0.05$); The cytotoxic test (OD value) was markedly higher in blocking group (0.29 ± 0.06) than that of control group (0.19 ± 0.09 , $p < 0.05$).